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Warming Seawater Temperature and Nutrient Depletion Alters Microbial Community Composition on a Foundational Canopy Kelp Species

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ABSTRACT

Warming seawater temperatures and low dissolved inorganic nitrogen (DIN) levels are environmental stressors that affect the health and abundance of marine macroalgae and their microbiomes. *Nereocystis luetkeana*, a canopy-forming species of brown algae that forms critical habitat along the Pacific coast, has declined in regions impacted by these synergistic stressors. Little is known about how these environmental factors affect the microbiome of *N. luetkeana*, which could affect nutrient availability, vitamin production, and stress response for the host. We experimentally tested the interactive effects of three seawater temperatures (13°C, 16°C, 21°C) crossed with abundant and replete DIN levels on the diversity and composition of blade-associated microbiomes from two spatially separated kelp host populations. We hypothesised that kelp microbiomes exposed to high temperatures and low DIN would experience the lowest diversity. Contrary to our hypothesis, the highest temperature treatment resulted in the largest increase in microbial diversity, and microbiomes in all temperature treatments experienced a decrease in previously dominant taxa. Temperature had a larger effect than DIN on the kelp microbiome in all cases. The disruption to the kelp microbiome across all temperatures, especially at the highest temperature, suggests that the effects of warming on *N. luetkeana* extend to the microbiome.

1 | Introduction

Kelp are an important foundational species in nearshore marine environments. Kelp forests create critical biodiversity hotspots (Steneck et al. 2002), are loci for coastal nutrient cycling (Pfister et al. 2019), and are a major contributor to carbon fixation (Wheeler and Druehl 1986; Krause-Jensen et al. 2018; Wilmers et al. 2012; Weigel and Pfister 2021). Their health and abundance are influenced by environmental variables associated with global climate change, such as water temperature and changing nitrogen concentrations in marine environments

(Krumhansl et al. 2016; Fales et al. 2023). These environmental variables further affect the diverse microbiome hosted on kelp tissue (Qiu et al. 2019).

Microbial taxa found on kelp may be selected for their ability to metabolise dissolved organic carbon kelp exuded by their host (Egan et al. 2013; Selvarajan et al. 2019; Weigel and Pfister 2019). This kelp microbiome is thought to be functionally important to the host (Weigel et al. 2022; Miranda et al. 2022; King, Moore et al. 2023a; Davis et al. 2023; Burgunter-Delamare et al. 2023), providing several benefits, including the provisioning of

nutrients and vitamins (Croft et al. 2005; Hochroth and Pfister 2024) and protection from pathogenic bacteria and infections (Li et al. 2022).

Nereocystis luetkeana, also known as bull kelp, is a canopy-forming kelp that grows from the Aleutian Islands in Alaska to central California. Highly abundant bacterial genera—such as *Mariniblastus* or *Granulosicoccus*—of the *N. luetkeana* blade microbiome possess genes necessary for nitrogen cycling and vitamin B12 synthesis (Weigel et al. 2022; Younker et al. 2024), activities that may benefit the kelp host. While bull kelp populations have historically been relatively stable and persistent at coastal sites in Washington state (Pfister et al. 2018), some populations have rapidly declined in recent years within the Salish Sea, including in the Puget Sound and the Strait of Georgia (Berry et al. 2021; Starko et al. 2022). These inland waters of the Salish Sea experience higher temperatures and lower dissolved inorganic nitrogen (DIN) concentrations than waters on the outer coast of Washington State (Berry et al. 2021; Hochroth and Pfister 2024). High temperatures have led to rapid declines in many kelp populations worldwide (Smale 2020), including *N. luetkeana* (Berry et al. 2021; Supratya et al. 2020), and abundant nitrogen is important for sustaining kelp growth, especially at high temperatures (Fernández et al. 2020). Kelp blade-associated microbes display significant variation across the geographical range of Washington State (Weigel and Pfister 2019), with evidence that microbiome abundance and diversity were reduced at the southernmost bull kelp population in the Salish Sea (Ramírez-Puebla et al. 2022). While the mechanisms driving these differences in community composition across geographic areas are yet unclear, salinity, temperature, and the health of the host kelp population are thought to play a role (Weigel and Pfister 2019; Florez et al. 2019; Ramírez-Puebla et al. 2022).

Increased seawater temperatures have significant impacts on marine microbiomes. High seawater temperatures are often correlated with coral bleaching, disease outbreaks, and functional change in coral microbiomes (Lima et al. 2020; Voolstra et al. 2024), and changes in the availability of nitrogen alter microbial community composition in the water column and on the host tissues of *Sargassum* species (Meyer-Reil and Köster 2000; Li et al. 2022). Many marine microbial taxa cannot grow at high water temperatures (MacLeod 1965; Huete-Stauffer et al. 2015), and slow-growing taxa can outcompete fast-growing taxa at high temperatures (Abreu et al. 2023). Increased temperature can drive dysbiosis, which is associated with reduced kelp growth in *Macrocystis pyrifera* (Minich et al. 2018) as well as an increase in the abundance of pathogenic taxa on *Ecklonia radiata* (Vadillo Vadillo Gonzalez et al. 2024). However, how microbial community structure responds to temperature can vary among individuals, such as on the rockweed *Fucus vesiculosus*, though the cause of inter-individual variability remains unknown (Stratil et al. 2013).

Nitrogen availability can impact algal microbiome composition, with particularly strong effects on nitrogen-fixing taxa. In *M. pyrifera* populations, a lack of available nitrogen can cause an increase in the abundance of ammonifying bacteria in the kelp-associated microbiomes (Florez et al. 2019, 2021). In one instance, nitrogen nutrient stress had a dominant effect over temperature on microbial community composition (Mancuso et al. 2023), while the combination of nutrient and temperature stress was relatively similar and not synergistic for the overall bacterial diversity or the resilience of the bacterial epibiota (Morrissey et al. 2021). Given increasing evidence of the microbiome's importance to its host's overall fitness, understanding how microbes respond to climate change-driven environmental stressors in the natural context of the host is a key aspect of understanding the resilience of a host species to our changing climate.

We experimentally tested how the *N. luetkeana* blade-associated microbiome responded to the interactive effects of seawater temperature and DIN availability. Using a controlled seawater mesocosm system, we crossed three seawater temperatures (13°C, 16°C, 21°C) with two DIN concentrations (high, $80\,\mu M$ vs. low, $<3\,\mu M$) to achieve 6 treatment conditions. Kelp blades were collected from two spatially separated populations—a population that experiences cold seawater and high flow conditions with higher nitrate concentrations (Turn Rock, WA, USA) and a contrasting population that experiences warmer seawater conditions and lower nitrate concentrations (Cherry Point, WA, USA) (Gierke et al. 2023). We quantified microbial community and diversity shifts on kelp blades across all six treatments.

We hypothesised that microbiome diversity would be negatively correlated with temperature and positively correlated with DIN, following patterns seen for kelp blade microbiomes in the wild (Ramírez-Puebla et al. 2022). Further, we hypothesised that blade-associated microbiomes from the warm water population (Cherry Point) and the cold water population (Turn Rock) would be initially different in composition, and that microbiomes from Cherry Point would be more resistant to changes in diversity and composition at higher seawater temperatures.

2 | Experimental Procedures

2.1 | Field Collection and Experimental Design

This study was conducted in tandem with Fales et al. (2023), which tested the physiological responses of the host kelp to temperature and nutrient stressors. Blades of *N. luetkeana* were collected on 6 July 2022 from Turn Rock, WA (48.5354°N, 122.9646°W), a cold and nutrient-rich site, and Cherry Point, WA (48.8618°N, 122.7482°W), a warm and nutrient-poor site (Fales et al. 2023). While seawater temperatures at Turn Rock rarely exceed 14°C, temperatures at Cherry Point exceeded 18°C on multiple days during 2022 (Weigel et al. 2023). Nitrate concentrations were higher at Turn Rock ($23.40 \pm 1.02\,\mu M$) compared with Cherry Point ($0.80 \pm 0.03\,\mu M$). Blades were transported in coolers back to Friday Harbour Laboratories, where seawater temperatures and nutrient concentrations were manipulated in 100 L recirculating tanks. There were six experimental treatments consisting of three temperatures (13°C, 16°C, 21°C) crossed with two nitrogen levels (high, $80\,\mu M$ vs. low, $<3\,\mu M$), where the high treatment exceeded usual concentrations, and the low treatment was depleted relative to ambient levels (Murray et al. 2015). Kelp blades were tagged with fishing line and plastic beads sewn into the base of the blade for identification, allowing for the tracking of individual blades over the incubation period. Blades of *N. luetkeana* were trimmed to 40 cm from the

base of the blade to standardise size, and all microbial sampling occurred after blade trimming. Details of the experimental design, tank conditions, and methods of temperature and nutrient manipulation are in Fales et al. (2023), where this experiment is referred to as the "population experiment." Briefly, 16 kelp blades ($n = 8$ per population per treatment) were incubated in 6 treatment tanks ($n = 3$ temperatures, $n = 2$ nutrient conditions), with blades of both populations mixed in each treatment tank. Submersible micro-pumps provided flow to ensure blade movement, and recirculating seawater was maintained at each treatment temperature with a system of chillers and heaters attached to external controllers. Natural seawater was UV-sterilised to deter phytoplankton and diatom growth prior to starting experiments, and water flow was independent in each tank. During the initial 5-day acclimation period, seawater temperatures were raised by 2°C per day until treatment temperatures were reached. Measured temperature means ($\pm$ SD) during the treatment period were 12.66 ± 0.17, 16.20 ± 0.11, and 20.63 ± 0.13. Seawater nutrients were manipulated by letting kelp blades draw down nutrient levels during the first 2 days, after which nutrients (NO_3, NH_4 and PO_4) were added daily using a 16:1 N:P ratio for both high and low N treatments to ensure that phosphorus did not become limiting. Nutrient means during the treatment period were: $78.6 \pm 30.9 \mu M$ NO_3, $1.1 \pm 0.3 \mu M$ NH_4, and $2.1 \pm 0.2 \mu M$ PO_4 (high N) vs. $2.5 \pm 0.5 \mu M$ NO_3, $0.3 \pm 0.1 \mu M$ NH_4, and $0.5 \pm 0.06 \mu M$ PO_4 (low N). Light levels were set to 100 μM PAR via LED lights on a 16:8 light–dark cycle.

2.2 | Sampling Blade Microbiomes

The surfaces of the kelp blades were sampled before placement into experimental tanks and sampled again after a 10-day experimental treatment, which consisted of a 5-day acclimation period followed by 5 days of treatment conditions. The blades of 10 individuals per treatment (5 individuals from each population) were swabbed for microbial analysis on one side of the blade with a sterile cotton swab for 20 s. The same individual blades were tracked and re-sampled at the end of the incubation with the same technique. To quantify sources of water column microbes, we collected seawater at Turn Rock and Cherry Point upon blade collection, from the initial source seawater in the incubation tanks, and from tank seawater following 10 days of incubation. We collected 3 replicates × 1 L samples of seawater from each site in separate acid-washed Nalgene bottles, and 2 replicates × 1 L seawater samples from the incoming seawater tanks. Finally, 1 L of water was collected from each of the 6 seawater tanks after the incubation period. All 14 total 1 L seawater samples were filtered through 0.22 μm Sterivex filters using a peristaltic pump, though only 500 mL was filtered for the three Cherry Point samples due to the presence of more particulates.

2.3 | DNA Extraction and Processing

DNA was extracted from the swabs using the QIAGEN DNeasy PowerSoil Pro Kit. To extract DNA from water samples filtered through 0.22 μm Sterivex filters, a PVC cutter and sterilised tweezers were used to extract half the filter paper and place it into a bead-beater tube for extraction following methods described in Pfister et al. (2019) and Jackrel et al. (2017). From each

temperature and nitrogen treatment, 8 samples (from the 10 blades sampled per treatment) were submitted for sequencing. DNA was amplified, sequenced, and amplicon sequence variants (ASVs) were identified by the Duchossois Family Institute Microbial Metagenomics Facility at the University of Chicago. Extracted DNA was PCR amplified for the V4–V5 region within the 16S ribosomal RNA (rRNA) using universal bacterial primers—563F and 926R. 2×250 paired-end read sequencing was performed on the Illumina MiSeq platform, using the QIASeq 1-step amplicon kit (Qiagen) for generating libraries. Of the 48 pre-incubation blade samples, 15 failed to amplify during PCR and were not sequenced, likely due to low DNA extraction yield. Of the 48 post-incubation blade samples, 3 failed to amplify during PCR and were not sequenced. All 14 seawater samples successfully amplified.

Raw 16S gene sequence data were processed into ASVs using the dada2 pipeline (Callahan et al. 2016) and following methods described in Younker et al. (2024). Reads were first trimmed at 210 bp for forward reads and at 150 bp for reverse reads to remove low-quality nucleotides. Chimaeras and singleton reads were detected and removed using the default consensus method. Then, ASVs with lengths between 320 and 365 bp were retained as high-quality ASVs. Taxonomy of the resultant ASVs was assigned to the genus level using the RDP classifier (v2.13) with a minimum bootstrap confidence score of 80. ASVs that were identified as chloroplasts were removed prior to analysis in R. Two samples that may have been initially contaminated with bacteria of gut, faecal, or non-marine origin during the sampling process were also removed, reducing the total number of samples to 46, though we recognise that they could have been in the nearby seawater due to anthropogenic sources (Korajkic et al. 2019; Orel et al. 2022). Although 16S rRNA generally results in unequal sequencing depths, we report analyses without rarefying ASV counts, so that we could capture all dynamics of colonisation and loss of taxa on individual kelp blades (McMurdie and Holmes 2014; Schloss 2023). Further, we tested the effects of rarefying ASVs and found that it did not change the outcome of our statistical analyses.

2.4 | Statistical Analyses

Microbial communities were analysed in R (Version 2023.03.0 + 386) to determine community composition, community diversity, and relative change in abundance of specific taxa. Alpha diversity indices (Shannon Diversity and observed ASV richness) were calculated (R package *phyloseq*, McMurdie and Holmes 2013) and their relationship to temperature, DIN, and sampling site were tested with analysis of variance (ANOVA) (R package *vegan*, Oksanen et al. 2013). We followed our ANOVA tests with post hoc statistical analyses (Tukey HSD) to assess significant differences between treatments (temperature, DIN, and sample site) within our samples. We visualised differences in microbiome composition (beta diversity) across treatments with principal coordinate analysis (PCoA) using the Bray-Curtis distance metric (R package *phyloseq*, McMurdie and Holmes 2013). Compositional differences in microbial communities were tested with permutational multivariate analysis of variance (PERMANOVA) (R package *vegan*, Oksanen et al. 2013). Alpha and beta diversity analyses were analysed at

both initial (T_0) and final (T_f) timepoints separately. To identify ASVs that were potential drivers of compositional differences, we conducted SIMPER analyses (R package *vegan*, Oksanen et al. 2013) and Log2FoldChange tests (R package *DESeq2*, Love et al. 2014) between temperature and nutrient treatments at the initial (T_0) and final (T_f) timepoints.

3 | Results

Our 16s rRNA amplicon sequencing of seawater and kelp-associated bacterial taxa generated between 406 and 68,391 reads per kelp blade, with a mean of 30,178 reads. At the beginning of the experiment (T_0), Turn Rock had higher ASV richness and Shannon Diversity (576 ASVs, Shannon = 2.825) than Cherry Point (382 ASVs, Shannon = 2.169) (Table S1), with 238 of these ASVs shared between the two sites (Figure S3). Bacterial community composition differed significantly between Cherry Point and Turn Rock (PERMANOVA, $t = 4.508$, $p = 0.004$) (Figure 1A): kelp blades from the Cherry Point population were dominated by Verrucomicrobia, while kelp blades from the Turn Rock population were dominated by Gammaproteobacteria (Figure 1C). At the genus level, kelp blade microbiomes sampled from Cherry Point were dominated by *Luteolibacter* and *Granulosicoccus*, while Turn Rock microbiomes showed high relative abundances of *Vibrio*, *Colwellia*, and *Granulosicoccus* (Figure 1C). Differences in the abundance

of *Luteolibacter*, *Rubritalea*, and *Persicirhabdus* contributed to 70% of the difference between the Turn Rock and Cherry Point microbiomes (Table S5); despite the high abundance of *Vibrio* in some Turn Rock samples, it did not significantly contribute to the differences between microbiomes.

At the end of the experiment (T_f), after 10 days of experimental manipulation, the alpha diversity of microbial communities from both sampling sites changed. Observed ASV Richness increased with temperature but did not significantly change under DIN enrichment or depletion (Table 1, Table S1). Shannon diversity was significantly affected by sampling site (ANOVA, $F_{1,33} = 22.486$, $p < 0.001$), temperature–DIN interaction (ANOVA, $F_{2,33} = 14.636$, $p = < 0.001$), and temperature–sample site interaction (ANOVA, $F_{2,33} = 6.524$, $p = 0.004$) (Table S2, Figures 2 and 3). Conversely, only temperature (ANOVA, $F_{2,33} = 9.482$, $p = 0.005$) had a significant effect on ASV richness (Figure 4A, Table S2). At the end of the experiment, both ASV richness and Shannon diversity were highest at 21°C (777 ASVs) compared to 16°C (629 ASVs) and 13°C (497 ASVs). There was no significant difference in ASV richness when comparing 13°C–16°C and 16°C–21°C, but ASV richness was significantly higher at 21°C than at 13°C (Figure 4, Table S3).

Temperature, DIN treatment, and their interaction all had a significant impact on kelp blade microbiome composition (PERMANOVA, $p < 0.001$, Table 1). While r^2 values were low,
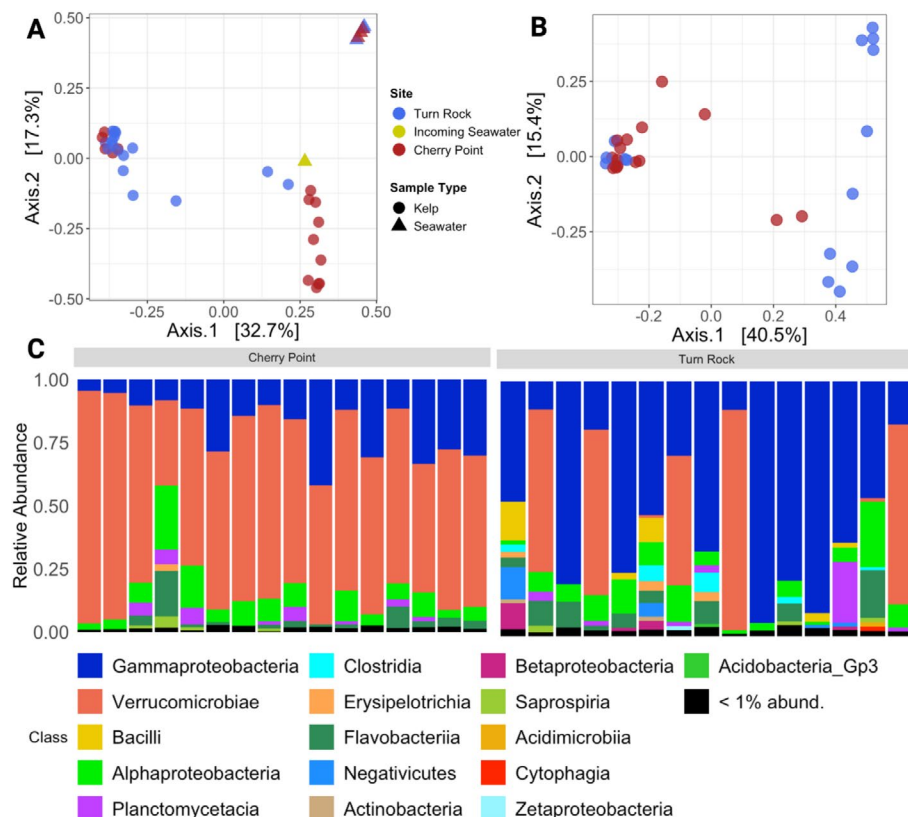

FIGURE 1 | Principal Coordinates Analysis (PCoA) of kelp blade microbial communities and the initial relative abundance prior to the experimental manipulation of temperature and nutrients. (A) PCoA of kelp blade microbiomes from Turn Rock and Cherry Point and seawater microbiomes from incoming seawater to the experimental mesocosms. Each point on the PCoA represents the microbiome of an individual kelp blade. (B) PCoA of initial kelp blade microbiomes grouped by the two source sites only. (C) The initial relative abundance of microbial classes at each site prior to the experimental manipulation of temperature and nitrogen.

TABLE 1 | The effect of temperature and nutrients on the Alpha Diversity (Shannon, Observed ASV Richness) of bacterial taxa in association with *Nereocystis luetkeana* from two different sites at the final sampling (T_f).

	Treatment	*F*-Value, Observed ASV Richness	*F*-Value, Shannon Diversity	R2, Bray-Curtis PERMANOVA	*F*, Bray-Curtis PERMANOVA	Degrees of Freedom
T_f	Temperature	**6.159 ****	0.087	0.076	**6.283 *****	2
	DIN	2.989	0.156	0.232	**9.646 *****	1
	Site	0.914	**1.556 *****	0.069	**5.714 *****	1
	Temp:DIN	1.701	**1.013 *****	0.051	**2.116 ****	2
	Temperature:Site	2.766	**0.452 ****	0.017	1.441	2
	DIN:Site	0.548	0.044	0.132	**5.492 *****	1
	Temperature:DIN:Site	2.345	**0.290 ***	0.026	1.090	2
T_f Cherry Point	Temperature	**4.279 ***	**0.005 ****	0.372	**8.769 *****	2
	Nitrogen	2.022	0.051	0.090	**4.23 *****	1
	Temp + DIN	2.971	**0.005 ****	0.179	**4.216 *****	1
T_f Turn Rock	Temperature	**4.123 ***	0.192	0.264	**4.422 *****	2
	DIN	0.978	0.725	0.093	**3.125 ****	1
	Temp + DIN	0.576	**0.001 ****	0.166	**2.789 *****	1

Note: ANOVAs are reported for the main effects of temperature and nutrients and their interaction. Beta Diversity differences were quantified with Bray-Curtis Distances and tested with PERMANOVA. Each treatment was replicated with 8 individual kelp blades from each site. Significance values are *** for $p < 0.001$, ** for $p < 0.01$, * for $p < 0.05$, and for $p < 0.01$. Significant p values are bolded.
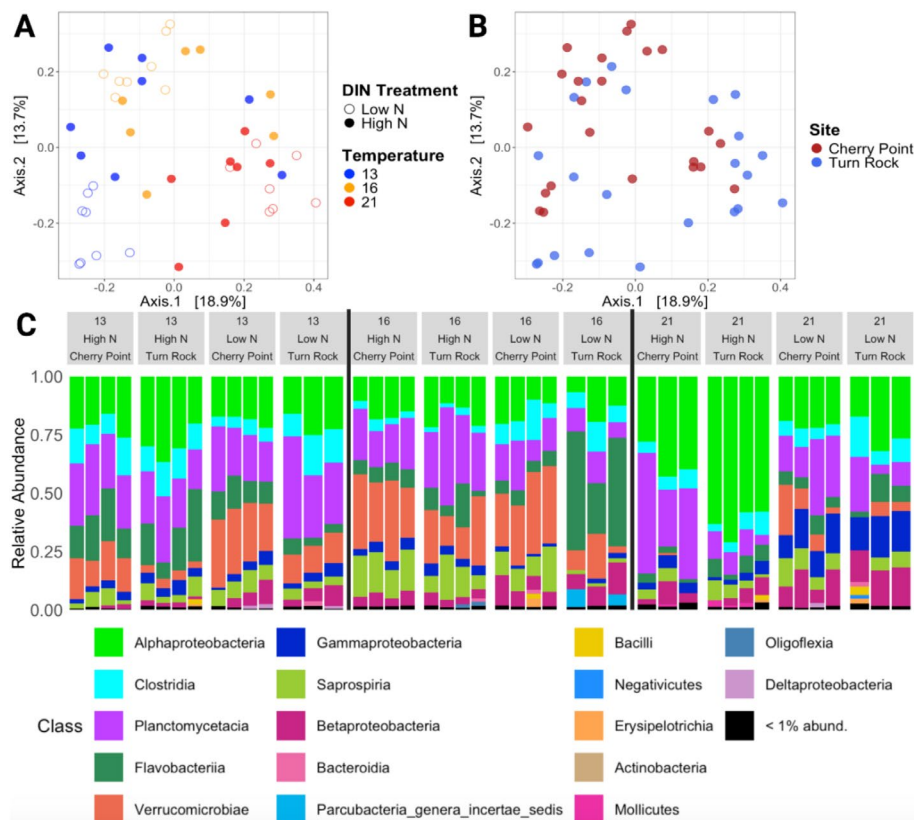

FIGURE 2 | Principal Coordinates Analysis (PCoA) of kelp blade microbial communities following temperature and nutrient manipulation. Each point on the PCoA represents the microbiome of a specific kelp blade. (A) Bacterial communities on kelp blades grouped by temperature and nitrogen treatments and (B) grouped by site only. (C) The relative abundance of bacterial classes in the kelp blade microbiome following experimental temperature and nitrogen manipulations.
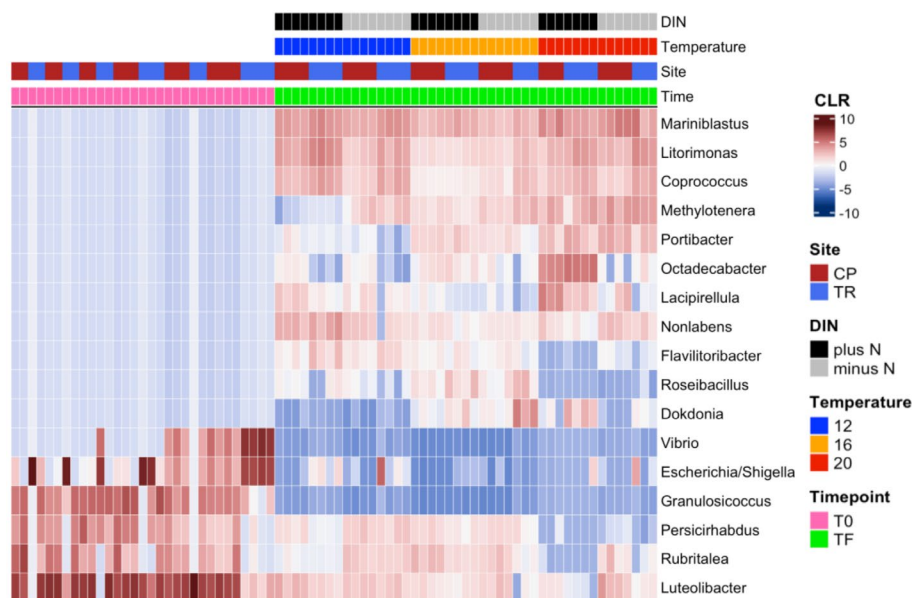
FIGURE 3 | Genus-level heatmap of the most abundant taxa at the beginning and end of the nitrogen and temperature manipulations. Abundances are centre log-ratio transformed. Shifts in the abundance of different genera are shown in Table S5.
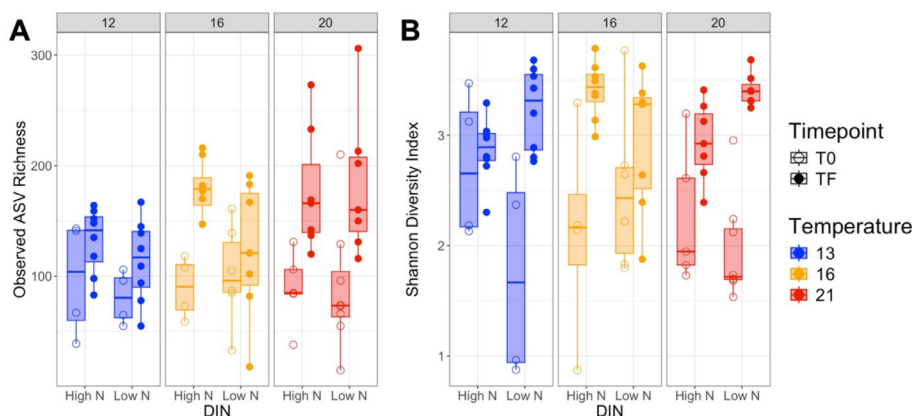

FIGURE 4 | (A) ASV richness and (B) Shannon Diversity Index of kelp-associated bacteria before (T_0) and after (T_f) the experimental manipulation of temperature and nitrogen. Each point is the ASV richness of an individual kelp blade microbiome.

temperature (PERMANOVA, $r^2 = 0.207$, $p = 0.001$) explained more of the variance in community composition than DIN (PERMANOVA, $r^2 = 0.064$, $p = 0.001$) or the interaction between temperature and DIN (PERMANOVA, $r^2 = 0.126$, $p = 0.001$) (Table 1). Microbial communities from temperature treatments 13°C and 21°C were compositionally distinct, while microbial taxa from temperature group 16°C partially overlapped with 13°C and 21°C treatments (Figure 2A). Cherry Point and Turn Rock kelp blade microbiomes remained significantly different in community composition at the end of the experiment (PERMANOVA, $F = 4.508$, $p = 0.002$) but did not display strong clustering by site at the end of the experiment (Figure 2B).

Across all six treatments, the most abundant genera before incubation decreased in abundance following incubation. The decrease in the most abundant genera was accompanied by an increase in other genera that were present in low abundance on the kelp blades before the experiment, with different patterns found across the temperature and DIN conditions (Figure 3).

The highest temperature treatment, 21°C, experienced the most change in abundance of individual ASVs compared to the 13°C and 16°C treatments, with 202 ASVs significantly differentially abundant compared to 56 ASVs and 23 ASVs, respectively (Figure 3, Table S5). Overall, more ASVs appeared in the kelp blade microbiome post-incubation (232 ASVs total) than disappeared from the kelp blade microbiome post-incubation (50 ASVs) (Table S5). *Luteolibacter*, *Granulosicoccus*, *Rubritalea*, *Persicirhabdus*, and *Vibrio* were highly abundant before incubation but decreased in relative abundance following incubation (Figures 1C and 2C, Figure S2). These genera remained present after incubation but did not dominate blades as they previously did (Figure 3). *Mariniblastus* and *Litorimonas* became the most consistently abundant genera after the incubation at all temperatures (Figure 3). *Octadecabacter* became highly abundant at 21°C in the DIN-depleted condition (Figure 3). Many additional genera were abundant post-incubation across several treatment regimes, including *Nonlabens*, *Portibacter*, and *Methylotenera* (Figure 3).

Kelp blade microbiomes were distinct in composition from the surrounding seawater at the start of the experimental incubation (PERMANOVA, $r^2 = 0.207$, $p = 0.001$), and seawater bacterial communities did not cluster with those from kelp blades in any temperature or nitrogen treatment at either of the sample sites (Figure 1A, Table S4). After the experimental incubation, kelp blade microbial communities remained distinct from seawater microbial communities sampled from treatment tanks (PERMANOVA, $r^2 = 0.167$, $p = 0.001$) (Figure S1). While there are genera of bacteria that are found in both the seawater and kelp microbiomes, only 1% of ASVs are shared between seawater and kelp, and our experimental manipulation did not alter this. ASVs from the phylum Firmicutes, many of which are common in faecal or gut microbiomes (i.e., *Coprococcus*, *Schnuerera*, *Escherichia* and *Shigella*) appeared on some kelp blades in relatively high abundance (Figure 3) but were not found in the filtered seawater or in the incoming tank seawater.

4 | Discussion

After 10 days of experimental manipulation, the composition and diversity of microbes associated with *Nereocystis luetkeana* responded to elevated temperatures and decreased nitrogen concentrations. Alpha diversity increased significantly at the highest temperature of 21°C, compared with more typical temperatures of 13°C, and community composition was significantly affected by site, DIN, and temperature. Of these three variables, temperature explained the most variation (around 20%) between microbial communities at the end of the experiment. An environmental variable explaining only 20% of compositional variation is relatively low but not novel to seaweeds (Stratil et al. 2013). The bacterial classes Verrucomicrobiae (mainly *Luteolibacter*) and Gammaproteobacteria (mainly *Granulosicoccus*) were dominant before the experiment, while the classes Alphaproteobacteria (mainly *Litorimonas* and *Octadecabacter*), Planctomycetes (mainly *Mariniblastus*), and Verrucomicrobiae (mainly *Rubritalea* and *Persicirhabdus*) were most abundant at the end of the experiment. Kelp blade microbiomes were distinct in composition from and shared few ASVs with seawater microbial communities, further lending evidence to the role of the kelp host as a strong filter of microbial taxa (Weigel and Pfister 2019; Liu et al. 2022; Michelou et al. 2013).

4.1 | Elevated Temperature Resulted in Increased Diversity and Shifts in Microbial Community Composition

Elevated seawater temperatures resulted in increases in microbial alpha diversity. The increased alpha diversity at high temperature contrasts with a previous study of wild *N. luetkeana* populations in the Salish Sea, where lower microbial abundance and diversity characterised a population exposed to high temperatures and low DIN (Ramírez-Puebla et al. 2022). However, temperature-induced increases in alpha diversity have been detected in corals (Maher et al. 2019) and in other kelp species in controlled incubation experiments (Minich et al. 2018; Vadillo Gonzalez et al. 2024). Our experimental conditions might have favoured fast-growing or generalist microbes such as the Alphaproteobacteria *Litorimonas*, one of the most abundant

genera after incubation. Alphaproteobacteria are common in algal microbiomes (Goecke et al. 2013) and have been characterised as fast-growing opportunistic generalists (Bengtsson et al. 2011). We acknowledge that changes to bacterial alpha diversity can result when using experimental mesocosms, either through stress-related bacterial species loss or through the colonisation of atypical species when enclosed with a host, and may differ from bacterial community changes in intact kelp in the marine environment.

Several initially low-abundance taxa increased in relative abundance in all temperature treatments throughout the experiment, alongside relative decreases in the most abundant taxa at the start of the experiment (Figure 3). The decreased dominance of a few genera and the increase in the abundance of many taxa, especially at high temperatures, could lead to an increased number of pathogenic microbes, as seen in other studies (Case et al. 2011; Vadillo Gonzalez et al. 2024). Macroalgae-associated microbiomes become vulnerable to invasion by pathogenic bacteria, demonstrated among diverse macroalgae such as *Cystoseira compressa* (Mancuso et al. 2023), *Delisea pulchra* (Case et al. 2011), and *Macrocystis pyrifera* (Minich et al. 2018). However, the genera that were found to be highly abundant after incubation have diverse functions and may not be strictly pathogenic. For example, the genus *Mariniblastus* became the most abundant across all temperatures, consistent with observations on the kelp *Ecklonia radiata* (Vadillo Gonzalez et al. 2024). *Mariniblastus* is thought to play a role in maintaining macroalgal biofilms and degrading macroalgal-produced polysaccharides (Faria et al. 2018). *Litorimonas*, which increased at 13°C, is thought to be an opportunistic pathogen on *Saccharina japonica* (Li et al. 2020) but is also present in similar abundances on healthy *S. japonica* individuals (Zhang et al. 2020) and in many other species of macroalgae (Park et al. 2022), including *Macrocystis pyrifera* (James et al. 2020). Other studies report that *Litorimonas* potentially plays a role in photosynthesis and is reported as a core taxon across many seaweed microbiomes (King, Uribe et al. 2023b).

The increase in specific bacterial taxa post-incubation, such as *Octadecabacter* and *Granulosicoccus,* could have positive effects on the kelp host if these colonists provide benefits such as B vitamins (Croft et al. 2005; Younker et al. 2024). *Octadecabacter* and *Granulosicoccus* both may produce vitamin B12 (Dogs et al. 2017; Weigel et al. 2022), and increases in *Octadecabacter* at 21°C could compensate for the loss of *Granulosicoccus* following host exposure to high temperatures. Finally, the Verrucomicrobiae *Luteolibacter* was one of the most abundant bacteria before incubation but declined after incubation in all treatments, especially at 21°C. Members of this class have antimicrobial properties on kelp tissues (Vollmers et al. 2017), and their decrease may have allowed previously suppressed taxa to increase. In this light, the relative decrease of the dominant microbial taxa throughout the course of the experiment may better explain the increase in diversity seen.

Regardless of the pathogenic or beneficial functions of the taxa that increased after incubation, the overall change in microbial community structure could be a sign of dysbiosis associated with environmental or host stressors. In the context of the experiments here, we define dysbiosis as a shift in the microbiome

that occurs with host stress. We assessed if post-incubation microbiomes were consistent with the Anna Karenina Principle, which states that dysbiotic microbiomes vary more in composition than healthy microbiomes (Zaneveld et al. 2017). This principle has been observed in coral microbiomes (McDevitt-Irwin et al. 2019) and macroalgal microbiomes (Bonthond et al. 2023). In our experiment, the increase in abundance of previously low-abundance genera at the highest temperature (21°C) was associated with increased kelp stress, including elevated respiration rates and extremely low growth rates (Fales et al. 2023). However, kelp blade microbiomes did not become significantly more dispersed in composition after incubation in our experiment, so they did not reveal dysbiosis as characterised by the Anna Karenina Principle (Table S6). An increase in alpha diversity as community composition becomes dissimilar could also be an indicator of dysbiosis (McDevitt-Irwin et al. 2019; Maher et al. 2019). Our experiment partially agrees with this, as Observed ASV richness did increase overall—most significantly at the highest temperature—but 21°C microbiomes did not diverge in composition significantly more than microbiomes at 13°C or 16°C (Table S6). While we can conclude that the structure of the microbiomes was significantly altered post-incubation, we cannot yet address whether there are functional consequences to the host, as there is little functional information on this kelp's microbiome. We do demonstrate, however, that an association between host and environmental stress changes the associated microbial community and note that continued feedback among the microbial community and host health is likely.

The effects of elevated temperatures were seen in both host performance traits and microbial community composition. The response of the host kelp to high temperatures (Fales et al. 2023) likely plays a role in the changes that we observed in the microbiome. Regardless of nitrogen levels, kelp blades in the highest temperature (21°C) treatments experienced noticeable stress with blade tissue decay, decreased growth rates, and increased respiration rates (Fales et al. 2023). When temperatures increase, algal host defences may be compromised (Wright et al. 2000; Egan et al. 2013), as is the case with furanone compound-based chemical defence production in macroalgae (Zozaya-Valdés et al. 2016). Further, decreases in secondary metabolite and chemical defence production are correlated with bacterial pathogen-induced kelp bleaching (Campbell et al. 2011). High temperatures were also correlated with increases in alpha and beta diversity in the microbiome of *Gracilaria vermiculophylla*, hypothesised to be the result of higher metabolic rates of the microbes or reduced host control of microbial community structure, including a decline in the secondary metabolites of the host (Bonthond et al. 2023). If these defences are reduced due to host stress at high temperatures, it could create an opportunity for taxa usually held at low abundance to increase in abundance, an outcome consistent with our results.

4.2 | DIN Had Limited Effects on Microbiome Composition and Alpha Diversity

Although temperature and concentrations of nitrogen often negatively covary in coastal marine ecosystems influenced by upwelling (Palacios et al. 2013), altered DIN availability was not as influential as temperature on *N. luetkeana* and its microbiome.

Instead, Fales et al. (2023) found strong responses in the kelp host to high temperature stress with minimal effects of low nitrogen levels, patterns which were mirrored here in the kelp microbiome. Responses to DIN could be taxon-specific, limiting its impact to bacterial taxa that primarily metabolise DIN compounds (Florez et al. 2019), and only if there are no further micronutrient limitations to bacterial metabolism (Kirchman et al. 2003). Nitrogen metabolisms are diverse in marine bacteria, and kelp-associated bacteria are also capable of metabolising dissolved organic nitrogen (Hochroth and Pfister 2024; Younker et al. 2024). Given the diversity of microbial nitrogen metabolisms and the feedback between the host kelp and its microbiome, it is likely that the kelp microbiome responds in species composition or in metabolic functions to nitrogen limitation, as has been demonstrated in other manipulations of nitrogen in mesocosm experiments (Florez et al. 2021). In contrast, temperature may have more immediate effects on both the host and associated microbes, with the effects of nitrogen availability taking longer to manifest than the timeframe of our experiment.

Nutrient and nutrient-temperature interactions remained a significant determinant of microbial community composition, but these patterns did not hold across all temperatures. Nutrient availability likely directly influenced the abundance of genera with nitrogen metabolisms, including *Mariniblastus*, *Granulosicoccus*, and *Octadecabacter* (Morrow et al. 2018; Weigel et al. 2022) which were more abundant in the high DIN treatments in our study (Figure 4, Table S5). However, the effect of nitrogen on abundance remained masked at specific temperatures. *Mariniblastus* decreased in abundance at 21°C regardless of DIN treatments, a trend that has been observed in studies of *Ecklonia radiata* (Vadillo Gonzalez et al. 2024). *Octadecabacter* only showed differential abundance with DIN treatment at 21°C, a temperature that is within the recorded optimal growth temperature for the genus (Park and Yoon 2014; Jin et al. 2023). Temperature-inhibited growth could mask the benefits that specific taxa gain from abundant environmental DIN.

4.3 | Source Population Remained a Significant Determinant of Microbiome Composition but Does Not Predict Response to Perturbations

Kelp blade microbiome composition differed between the two source populations (Cherry Point vs. Turn Rock, Figure 1B), and these site-based differences remained even after co-incubation of kelp blades from the two populations together in treatment tanks (Figure 2B). Before incubation, Cherry Point microbiomes were dominated by Verrucomicrobiae, and Turn Rock microbiomes were dominated by Gammaproteobacteria. Differentiation across sites by different bacterial classes was also found in a larger-scale geographic survey of *N. luetkeana* populations in Washington State, with abundant Verrucomicrobia found on kelp in warmer seawater locations in the inland Puget Sound and abundant Gammaproteobacteria found on kelp populations in cooler coastal seawater (Weigel and Pfister 2019). Site specificity in seaweed-associated microbiomes has been recorded elsewhere (Egan et al. 2013; Lachnit et al. 2009), and site effects were persistent following co-incubation in previous studies with *N. luetkeana* (Chen and Parfrey 2018). Furthermore, studies of giant kelp *M. pyrifera* populations in Chile found that

microbiomes of kelp sampled from different populations responded differently to temperature (Florez et al. 2019). While we did not correlate host performance to microbiome characteristics in this experiment, there were differences in the performance of the kelp from each population: *N. luetkeana* from Turn Rock grew significantly more in biomass, while *N. luetkeana* from Cherry Point grew significantly more in total blade area (Fales et al. 2023). These physiological differences between populations could play a role in how the microbiome responds to the environmental variables tested, but this is outside the scope of this paper.

While the source population remains an important factor in determining differences in microbial community structure, we did not find that Cherry Point microbiomes were more resistant to changes in diversity due to temperature and nitrogen manipulations. Cherry Point microbiomes experienced a greater overall increase in observed ASV richness and Shannon diversity (Table S1) and an increase in dispersion over the course of the experiment (Table S6), yet none of these changes were significantly different from Turn Rock (Table S6). In fact, Turn Rock microbiomes were significantly less dispersed at the end of the experiment (Table S6), unlike Cherry Point. However, due to a lack of significant results, we did not find evidence that Cherry Point microbiomes were more resilient to changing temperature and nitrogen conditions than Turn Rock microbiomes.

5 | Conclusions

Elevated seawater temperatures and decreased nitrogen availability can influence both the algal host and its microbiome (Minich et al. 2018; Vadillo Gonzalez et al. 2024; Florez et al. 2019), often with negative consequences for fitness and population persistence. Kelp populations are likely to face these environmental stressors in the future under climate change conditions in the Salish Sea (Khangaonkar et al. 2019) and elsewhere worldwide (Werner et al. 2016). Through experimental manipulation of seawater temperature and DIN, we found that elevated temperatures greatly altered the microbiome associated with *N. luetkeana* and that elevated temperature had stronger effects than manipulations of DIN, like the results on kelp host performance reported in Fales et al. (2023). At the highest temperature (21°C), we observed increased microbiome diversity driven by declines in the relative abundance of taxa thought to be beneficial to the host, coupled with the growth of many previously low-abundance bacterial taxa, which is a potential sign of microbiome dysbiosis. How the change in the kelp blade microbial community affects its function is unclear and requires more research. While seawater DIN depletion contributed less to compositional changes in the microbiome than elevated temperatures, we found significant effects of DIN on taxa with nitrogen-transforming metabolisms. We found evidence to support the importance of population-level variation in determining microbial community structure, even after environmental stress is applied. Overall, we show that high temperatures can alter kelp-associated microbiomes, underscoring the continued vulnerability of canopy kelp species to warming coastal waters.

Author Contributions

Nichos B. Molnar: conceptualization, writing – original draft, writing – review and editing, visualization, data curation, formal analysis, methodology, investigation. **Brooke L. Weigel:** methodology, writing – review and editing, conceptualization, investigation, funding acquisition, resources. **Robin J. Fales:** methodology, writing – review and editing, conceptualization, investigation, funding acquisition, resources. **Catherine A. Pfister:** conceptualization, funding acquisition, writing – review and editing, supervision, resources.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/, reference number KIHV01000000.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.